

transgene mRNA can then be produced by splicing between these two vector encoded sequences within circular concatamers.

The paragraph beginning at page 79, line 29 is amended as follows:

Intermolecular recombination of rAAV genomes to form single circular episomes may be particularly useful for gene therapy. For example, large regulatory elements and genes beyond the packaging capacity of rAAV may become linked after co-infecting tissue with two independent vectors (Figure 19). This strategy could also involve [trans-splicing] splicing of transcripts from vectors encoding two independent regions of a gene which are brought together to form an intact splicing unit by circular concatamerization.

In the Claims

Please cancel claims 1-39 without prejudice.

Please add the following new claims:

40. (New) A composition comprising:

- B4
- a) a first adeno-associated virus vector comprising linked:
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a portion of a gene which includes a transcriptional regulatory region;
 - iii) a third nucleic acid segment comprising a splice donor site; and
 - iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus; and
 - b) a second adeno-associated virus vector comprising linked:
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-

associated virus;

- ii) a second nucleic acid segment comprising a splice acceptor site;
- iii) a third nucleic acid segment comprising a portion of a gene which together with the nucleic acid segment of a)(ii) comprises a gene comprising an open reading frame which encodes a functional polypeptide; and
- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus.

41. (New) The composition of claim 40 further comprising a delivery vehicle.

42. (New) The composition of claim 40 wherein the nucleic acid segment of a)ii) comprises a promoter.

43. (New) The composition of claim 40 wherein the nucleic acid segment of a)ii) comprises an enhancer.

44. (New) A method to express a polypeptide in a host cell comprising contacting the host cell with the composition of claim 40 so as to express the functional polypeptide.

45. (New) A method to express a polypeptide in a host cell, comprising: contacting a host cell comprising a first adeno-associated virus vector comprising linked:

- a) i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
- ii) a second nucleic acid segment comprising a portion of a gene which includes a transcriptional regulatory region;

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- iii) a third nucleic acid segment comprising a splice donor site; and
- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

with a second adeno-associated virus vector comprising linked:

- B4
- b)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a splice acceptor site;
 - iii) a third nucleic acid segment comprising a portion of a gene which together with the nucleic acid segment of a)ii) comprises a gene comprising an open reading frame which encodes a functional polypeptide; and
 - iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

so as to yield a host cell which expresses the functional polypeptide.

46. (New) A method to express a polypeptide in a host cell, comprising: contacting a host cell comprising a first adeno-associated virus vector comprising linked:

- a)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a splice acceptor site;
 - iii) a third nucleic acid segment comprising a portion of a gene; and
 - iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

with a second adeno-associated virus vector comprising linked:

- b)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;

- ii) a second nucleic acid segment comprising a portion of a gene which includes a transcriptional regulatory region and which together with the nucleic acid segment of a)iii) comprises a gene comprising an open reading frame which encodes a functional polypeptide;
- iii) a third nucleic acid segment comprising a splice donor site; and
- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

so as to yield a host cell which expresses the functional polypeptide.

47. (New) A method to express a polypeptide in a host cell, comprising: contacting a host cell with a first adeno-associated virus vector and a second adeno-associated virus vector, wherein the first adeno-associated virus vector comprises linked:

- a)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a portion of a gene which includes a transcriptional regulatory region;
 - iii) a third nucleic acid segment comprising a splice donor site; and
 - iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

wherein the second adeno-associated virus vector comprises linked:

- b)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a splice acceptor site;
 - iii) a third nucleic acid segment comprising a portion of a gene which together with the nucleic acid segment of a)ii) comprises a gene comprising an open reading

frame which encodes a functional polypeptide; and

- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

so as to yield a host cell which expresses the functional polypeptide.

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48. (New) The method of claim 45 or 47 wherein the nucleic acid segment of a)ii) comprises a promoter.
49. (New) The method of claim 45 or 47 wherein the nucleic acid segment of a)ii) comprises an enhancer.
50. (New) The method of claim 46 wherein the nucleic acid segment of b)ii) comprises a promoter.
51. (New) The method of claim 46 wherein the nucleic acid segment of b)ii) comprises an enhancer.
52. (New) The method of claim 44, 45, 46 or 47 wherein the host cell is a muscle cell, brain cell, retinal cell, liver cell, lung cell or hematopoietic cell.
53. (New) The method of claim 44, 45, 46 or 47 wherein the polypeptide is the cystic fibrosis transmembrane receptor, polypeptide, β -globin, γ -globin, tyrosine hydroxylase, glucocerebrosidase, arylsulfatase A, factor VIII, dystrophin, or erythropoietin.
54. (New) The method of claim 44, 45, 46 or 47 wherein the host cell is a mammalian cell.

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55. (New) The method of claim 54 wherein the host cell is a muscle cell, brain cell, retinal cell, liver cell, lung cell or hematopoietic cell.

56. (New) The method of claim 44, 45, 46 or 47 wherein the host cell is selected from the group consisting of an avian cell, a bovine cell, a swine cell, an equine cell, an ovine cell, a canine cell, a feline cell, an amphibian cell, a reptilian cell and a fish cell.

57. (New) The method of claim 56 wherein the host cell is a muscle cell, brain cell, retinal cell, liver cell, lung cell or hematopoietic cell.

58. (New) The method of claim 44, 45, 46, or 47 wherein the vectors comprise DNA.

59. (New) A composition comprising:

a first adeno-associated virus vector comprising linked:

- a) i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
- ii) a second nucleic acid segment comprising a portion of a gene which includes a transcriptional regulatory region;
- iii) a third nucleic acid segment comprising a splice donor site; and
- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

which first vector, in the presence of a second adeno-associated virus vector comprising linked:

- b) i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
- ii) a second nucleic acid segment comprising a splice acceptor site;

- iii) a third nucleic acid segment comprising a portion of a gene which together with the nucleic acid segment of a)ii) comprises a gene comprising an open reading frame which encodes a functional polypeptide;
- iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

in a host cell yields a RNA transcript which comprises sequences from the first adeno-associated virus vector linked to sequences from the second adeno-associated virus vector, which sequences are positioned so that the splice donor site is 5' to the splice acceptor site, and which transcript is spliced to a mRNA which encodes the functional protein.

60. (New) A composition comprising:

a first adeno-associated virus vector comprising linked:

- a)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a splice acceptor site;
 - iii) a third nucleic acid segment comprising a portion of a gene; and
 - iv) a fourth nucleic acid segment comprising a 3'-inverted terminal repeat of adeno-associated virus;

which first vector, in the presence of a second adeno-associated virus vector comprising linked:

- b)
 - i) a first nucleic acid segment comprising a 5'-inverted terminal repeat of adeno-associated virus;
 - ii) a second nucleic acid segment comprising a portion of a gene which together with the nucleic acid segment of a)iii) comprises a gene comprising an open reading frame which encodes a functional polypeptide, wherein the portion of the gene of b)ii) includes a transcriptional regulatory region;